

# Analysis of trace element in intervertebral disc by Atomic Absorption Spectrometry techniques in degenerative disc disease in the Polish population

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## Abstract

**Objective.** Although trace elements are regarded crucial and their content has been determined in number of tissue there are only few papers addressing this problem in intervertebral disc in humans. Most of the trace elements are important substrates of enzymes influencing metabolism and senescence process. Others are markers of environmental pollution. Therefore the aim of the research was to analyzed of the trace element content in the intervertebral disc, which may be a vital argument recognizing the background of degenerative changes to be the effect of the environment or metabolic factors.

**Materials and methods.** Material consist of 18 intervertebral disc from 15 patients, acquired in surgical procedure of due to the degenerative disease with Atomic Absorption Spectrometry content of Al, Cd, Co, Pb, Cu, Ni, Mo, Mg, Zn was evaluated.

**Results.** Only 4 of the trace elements were detected in all samples. The correlation analysis showed significant positive age correlation with Al and negative in case of Co. Among elements significant positive correlation was observed between Al/Pb, Co/Mo, Al/Mg, Al/Zn Pb/Zn and Mg/Zn. Negative correlation was observed in Al/Co, Cd/Mg, Co/Mg, Mo/Mg, Co/Zn and Mo/Zn.

**Conclusions.** This study is the first to our knowledge that profiles the elements in intervertebral disc in patients with degenerative changes. We have confirmed significant differences between the trace element contents in intervertebral disc and other tissue. It can be ground for further investigation.

## Key words

environment, trace element, pollution, degeneration

## INTRODUCTION AND OBJECTIVE

Trace elements (TE) are considered to be an integral part of biological tissue. In chemical understanding, the concentration of elements is defined as being less than 100 µg/kg of the substance. In the biological sense, it is defined as dietary minerals needed in small quantities for proper physiology and development of the organism.

Although a numbers of studies have been performed there is still no full biological role definition, nor full consent for a complete list for humans. It has been shown that some of them are an inherent part of the metabolic process (copper, magnesium, zinc), while others are attributed as the side-effect of live cumulation due to environmental factors (lead, nickel, cobalt, cadmium). Therefore, analysis of TE may be suitable not only for for pollution monitoring, but also can

improve our understanding of the metabolic changes in selected tissues.

The majority of the analyses concern bone tissue, which is regarded as the trace element repository, reflecting its turnover in the whole organism [1]. Apart from that, new studies are connecting TE with the metabolic dysfunction of bone [2]. For selected purposes, the TE content may be evaluated in more unstable areas, such as serum, urine or cerebro-spinal fluid.

Intervertebral disc in humans (IVD) is highly specific tissue. Since it loses direct vascularisation in the first decade of life, all metabolism relies on the passive nutrient transport through the endplates becoming the natural barriers separating it from other tissues. This, in turn, determines the specific environment of the nucleus pulposus of IVD which is characterised by increased lactic acid concentration and low oxygen availability for the cells, resulting in the slowest metabolism compared to other tissues. The mechanical role of the IVD is to transmit and distribute high axial loads in the healthy spine withstanding the extortionate hydrostatic pressure. As the degeneration changes develop, the

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dysfunction of the extracellular matrix (ECM) is observed, resulting in the inability to accumulate water within the IVD to preserve mechanical function. Degenerative disc disease (DDD) is a complex and not fully understood entity. As an explanation for the disease, a number of theories have been proposed, including: aging, and genetic and environmental factors. It has been proved that an important role of the degeneration process is influenced by the disproportion of anabolic and catabolic activity of the ECM enzymes, such as cathepsins, aggrecanases, and matrix metalloproteinases (MMPs) [3]. The latter group of enzymes are zinc-dependant endopeptidase active in protein degradation, as well as being able to form other bioactive compounds [4]. Also, other TE, in chemical sense, are part of enzymes (among others, cytochrome oxidase, caeruloplasmine) or are involved in collagen synthesis, as well as being essential for connective tissue development [5].

TE analysis in the IVD may be suitable for several reasons. Since it may be considered as the most inert area, compared with other tissues, the cross-reference analysis with bone TE concentration may be more accurate in the prediction of timing and dose of environmental exposure. Additionally, the specificity of the nutrient transport through the end plate of environmentally-related TE concentration can improve our knowledge of end plate function and its possible role in DDD. Understanding the cross-relation of the metabolic-bound TE (Mg, Zn, Cu) will also change our knowledge of metabolic changes in the senescence and degeneration processes.

Although information from TE concentration in the disc is important, for the reasons presented above, there are only a few papers addressing this problem in humans. In the only study of a systematic character by Tahno et al. [6], the minerals evaluated (Ca, P, S and Na) do not meet the criteria for TE in the chemical or biological sense.

As mentioned before, bone is considered the primary site of exogenous elements to be stored. In platinum-treated patients with an ovarian tumour, it has been shown that the intradiscal metal accumulation may exceed up to 4.3-fold that observed in the vertebral body [7]. On incidental observation, metal detection was dependent on the administration route and, in some cases, was higher in disc and bone tissue than in internal organs, such as the liver and kidneys [8]. The presented study indicates the potential role of the intervertebral disc as a reference marker tissue.

**Objective.** The aims of the study were: (1) to develop a methodology for sample preparation and mineralisation for determination of TE by AAS analytical techniques; (2) to optimize the GF-AAS analytical technique for determination of elements in IVD samples; (3) to define the profile of the selected trace elements set in degenerated intervertebral disc in humans.

## MATERIALS AND METHOD

For use in the study, material from 18 intervertebral discs from 15 patients was obtained during surgical procedure. Six specimens were from cervical and 12 are lumbar spine, acquired during the surgical procedure of microdiscectomy, or spine fusion due to the degenerative disc disease. The mean age of the patients during the operation was 47.7,

ranging from 28–69 years. All patients were interviewed using a questionnaire to collect data on demography, health status, and occupational heavy metals exposure. None of the patients had knowledge of being inadvertently exposed to heavy metal pollution.

The use of tissue in the investigations was permitted by the Bioethical Committee of the Institute of Rheumatology in Warsaw on 31 May 2012, and the Bioethical Committee of the University of Medical Sciences in Poznań (No. 406/13).

The specimens were analyzed with Atomic Absorption Spectrometry (AAS) for trace elements concentration, and the concentration of subsequent elements evaluated: Al, Cd, Co, Pb, Cu, Ni, Mo, Mg, Zn. The concentration of trace elements were calculated for the dry weight (dw) of the disc.

**Analytical procedure.** The frozen intervertebral disc samples were freeze-dried for 24 hours using a Lyovac lyophilizer GT2e (Steris, Germany). After drying, the sample were weighed and nitric acid (suprapur, Merck, Germany) was added to obtain dilution factor (DF) of 10. The prepared samples were allowed to stand overnight in order to slow mineralization. The samples were then mineralized in a microwave oven (Mars Xpress 5, CEM, USA).

The concentrations of Al, Co, Cu, Cd, Mo, Ni and Pb were determined in 3 replications using an AAS 7000 spectrometer (Shimadzu, Japan) with graphite furnace atomisation (GF-AAS). The % RSD for GF-AAS analytical technique did not exceed 5%. The concentrations of Mg and Zn were determined in 3 replications with the use of Shimadzu AAS 7000 (Shimadzu, Japan) with flame atomization (F-AAS). The % RSD for F-AAS analytical technique did not exceed 7%. Table 1 presents basic optimised parameters of determination of Al, Co, Cu, Cd, Mo, Ni, Pb, Zn and Mg by the AAS analytical technique.

**Statistical analysis.** From the data mean the range of data was established. Where applicable, standard deviation (SD) was calculated. Spearman's rank-order correlation analysis was used to determine the relationship among different parameters. The  $p < 0.05$  was accepted as statistically significant. Statistica (Statsoft, Tulsa, OK) software was used for statistical analysis.

## RESULTS

In the proposed TE set, only 4 trace elements were detected in every sample: Al, Cu, Mg and Zn. The rest of the elements were observed in 16.65% (Mo) – 44.44% (Cd) of the samples. In the remaining samples, the concentration of those elements was below the level of detection for the method (Tab. 2).

The concentration of Mg, Zn and Cu was by one order of magnitude higher compared to the rest of examined elements (mg/kg dw vs µg/kg dw, respectively).

The content of elements determined in all samples was as follows (range values with mean value and standard deviation, respectively): Al: 440.2–1504, 775.1, 70.2 µg/kg dw, Cu: 0.97–5.6, 2.0, 2.9 mg/kg dw, Mg: 371.4–2132.0, 949.1, 48.7 mg/kg dw, Zn: 12.65–121.8, 39.9, 13.1 mg/kg dw.

For the remaining elements measured, only in a part of the samples the range values with mean values were as follows: Cd: 3.1–21.7, 8.6 µg/kg dw, Co: 2.6–294.2, 136.4 µg/kg dw,

**Table 1.** Basic optimised parameters of determination of Al, Co, Cu, Cd, Mo, Ni, Pb, Zn and Mg by GF-AAS and F-AAS analytical techniques

Parameter		Al	Cd	Co	Cu	Mo	Ni	Pb	Zn*	Mg*
Wavelength	[nm]	309,3	228,8	240,7	324,8	313,3	232	283,3	213,9	285,2
Slit	[nm]	0,7	0,7	0,2	0,7	0,7	0,2	0,7	0,7	0,7
Lamp current	[mA]	10	8	10	8	10	12	10	5	8
Lamp mode	BGC	SR	D2	D2	D2	D2	D2	D2	D2	D2
Drying	[OC]	150	150	150	150	150	120	150	-	-
Drying time	[s]	45	30	30	30	30	25	30	-	-
Ashing	[OC]	1000	500	400	800	1000	900	800	-	-
Ashing time	[s]	25	20	20	20	20	20	20	-	-
Atomization	[OC]	2600	2200	2500	2300	2600	2600	2400	-	-
Atomization time	[s]	5	5	5	5	5	6	5	-	-
Cleaning	[OC]	2700	2400	2700	2500	2700	2700	2600	-	-
Clearing time	[s]	2	2	2	2	2	2	2	-	-
LOD	[µg kg <sup>-1</sup> ]	<2.0	<0.04	<0.1	<0.3	<2.0	<0.04	<0.5	<0.1**	<0.03**

\*air-C<sub>2</sub>H<sub>2</sub> atomisation mode; \*\* LOD in [mg kg<sup>-1</sup>]

**Table 2.** Age of the patients and contents of metals in intervertebral disc. For Cd, Co, Pb, Ni and Mo limit of detection was given in samples were no TE was confirmed by the method used in the study

Patient code	Age	Al	Cd	Co	Pb	Ni	Mo	Cu	Mg	Zn
				[µg/kg dw]				[mg/kg dw]		
1	49	539.6	4.902	239.6	<5	<15	67.46	5.602	413.9	18.29
2	47	1504	<0.4	<1	18.35	92.09	<20	2.492	1195	61.26
2	47	915.1	21.72	145.2	<5	69.19	26.48	2.793	489.5	16.88
3	53	1103	<0.4	<1	24.09	19.25	<20	2.092	1599	71.22
3	53	1209	<0.4	<1	98.64	<15	<20	1.844	854.7	36.12
3	53	1127	<0.4	<1	<5	36.68	<20	1.889	2132	121.75
4	61	572.7	10.74	<1	<5	16.13	<20	1.459	672.1	25.72
5	64	1003	<0.4	<1	<5	<15	<20	0.9701	1622	29.38
6	57	1272	<0.4	<1	646.89	<15	<20	2.317	1475	64.62
7	38	451.2	4.903	11.74	<5	<15	<20	1.079	607.7	18.29
8	28	539.6	<0.4	294.2	<5	<15	<20	1.326	634.8	12.65
9	29	552.2	<0.4	2.611	<5	<15	<20	1.542	1008	38.97
10	49	470.2	3.111	182.8	<5	<15	40.92	1.616	371.4	14.06
11	52	520.7	<0.4	<1	9.181	197.7	<20	1.664	1032	55.76
12	43	697.4	8.501	<1	<5	<15	<20	1.799	516.7	34.35
13	39	490.7	6.511	<1	<5	<15	<20	2.046	977.9	43.84
14	49	545.9	<0.4	<1	<5	<15	<20	2.519	999.4	18.53
15	47	440.2	8.402	78.72	<5	<15	<20	1.417	482.8	36.78

Pb: 9.2–646.9, 159.4 µg/kg dw, Ni: 16.1–197.7, 71.8 µg/kg dw, Mo: 26.5–67.5, 44.9 µg/kg dw. (Tab. 2).

Correlation analysis showed significant positive age correlation with Al and negative in case of Co (Tab. 3). In mutual trace the correlation was significant for positive, bound in Al/Pb, Co/Mo, Al/Mg, Al/Zn Pb/Zn and Mg/Zn data pairs. A significant negative correlation was shown in Al/Co, Cd/Mg, Co/Mg, Mo/Mg, Co/Zn and Mo/Zn (Tab. 3).

DISCUSSION

This study is the first to show the selected trace element concentration in IVD, except for magnesium [6]. The study by Tohno et al., not only focused most attention on the element

**Table 3.** Spearman’s rank correlation between metals concentrations and metals concentrations with age in the IVD

	Al	Cd	Co	Pb	Cu	Ni	Mo	Mg	Zn	Age
Al	x	-0.34	-0.55*	0.47*	0.4	0.36	-0.22	0.62*	0.48*	0.49*
Cd		x	0.27	-0.33	0.06	0.03	0.38	-0.69*	-0.37	-0.26
Co			x	-0.35	-0.14	-0.29	0.64*	-0.72*	-0.7*	-0.55*
Pb				x	0.41	0.32	-0.2	0.29	0.53*	0.21
Cu					x	0.3	0.39	0.04	0.22	0.05
Ni						x	-0.02	0.36	0.39	0.22
Mo							x	-0.62*	-0.54*	-0.07
Mg								x	0.74*	0.47
Zn									x	0.37

\*Significant p<0.05

presented abundantly in the IVD structure, but also did not consider the generation process in the analysis. Taking into account the lack of data referring this particular biological area, it is possible only to refer to the presented findings of TE concentration in other tissues that have been studied so far. From the set of tissues, bone cartilage and tendons seem to be most relevant due to the metabolic similarities, morphological structure, as well as the biomechanical role in the human body [9, 10].

**Mg.** Magnesium levels have been well-documented in a variety of tissues, including intervertebral disc, as well as the temporomandibular joint disc (TMJD), which in the morphologic sense resembles the structure of the IVD [11, 12, 13, 14]. In research by Tohno et al. [6], the Mg level was determined at almost all levels in the spine, from C2 to S1 vertebrae. The analysis was performed by utilizing 9 specimens acquired *post mortem*, with no definition of the degeneration process. The average Mg content in the group was 1.196, ranging from 0.6 up to 2.2 mg/g, which is in accordance with the presented study – 949.1, ranging 371.4–2132 mg/kg dw (all units are cited as in the source articles). Unfortunately, this is the only element determined in both the Tohno et al. research and the presented study. To the best of the knowledge of the authors of the current study, no other studies exist which evaluate the elements in intervertebral disc tissue in humans.

Similar values were observed in examination of TMJD [13]. In the Takano et al. study, the level of the Mg in discs were slightly different compared to this study. The average value in the Takano et al. study was nearly half of the value detected in the current study – 524.74 µg/g vs. 949.11 mg/kg dw, respectively. The ranges observed were also wider in the current study – 393.4–764.9 µg/g dw vs. 371.4–2132 mg/kg dw, respectively. Similar values for Mg were also reported in the posterior longitudinal ligaments (PLL) of cervical spine 445 µg/g, [12], but the dispersion characteristic was almost 1/3 smaller compared to the presented study – 161 µg/g and 494.8 mg/kg of SD, respectively.

Compared to IVD, the average concentration of Mg in bones may be more than twice as high – 1792.9 µg/g [11]. In soft tissue of stomach, the concentration seems to be the lowest in examined tissues, ranging 30–300 mg/kg dw [15].

**Cu.** The Cu concentration was best documented in bone tissue. For Cu in bone, the average bone content was 0.62 µg/g dw [11] or 0.8 mg/kg dw [16].

Cu concentration in bone was not very different from cartilage, where it was found to be, on average, 0.79 mg/kg dw, with range within 0.20–1.78 mg/kg [16]. Compared to the above, the presented results show the level of copper in IVD to be up to twice as high than that observed in bone and cartilage (2.02, range 0.97–5.6 mg/kg dw).

**Zn.** Zinc concentration in intervertebral disc determined in the current study was similar to levels encountered in PLL by Kumai et al. (39.9 mg/kg dw and 36 µg/g dw, respectively) [17]. The average concentration in cartilage and bone was found to be more than twice as high – 88.3 (range 54.3–163.8) mg/kg dw and 84.58 µg/g (SD 17.68) µg/g, respectively [11, 16]. Again, the smallest concentration range was observed in stomach cells – 16–30 mg/kg dw [15].

**Al.** The determination of aluminium in all of the samples in the study was very interesting. The metal is considered to be non-essential but can be composed of highly reactive biomolecules. Although its role is not entirely known, brain tissue is considered the reservoir for systemic aluminium, and a relationship has been proved with neurodegenerative disorders [18]. The source is purely exogenic. For the purposes of the presented study, the literature is poor in the analysis of aluminium in tissues; therefore, this study presents new information that seems to be important for further analysis.

The rest of the elements evaluated in this study were determined with variable frequency: cadmium was detected in 44.4% (8 cases), cobalt 38.9% (7 cases), lead and nickel 33.3% (6 cases), and molybdenum in 16.67% (3 cases). In the remaining samples, the concentration was below the limit of detection.

**Cd.** Cd concentration in bones was detected in concentration up to 3 times higher compared to that in discs – 0.03 µg/g dw [11] vs. 7.5 µg/kg dw, respectively. Although the presented study reveals only the SD value, it is not certain that the element was detected in all specimens. Also, Łanocha et al. [19] revealed a higher concentration for Cd in bone tissue (average and range 0.22, 0.013–0.034 mg/kg dw). In a population occupationally-exposed to Cd, the bone concentration can rise and range from 0.11–1.2 mg/kg dw [20]. The cartilage Cd concentration is congruous with the levels observed in bone [16] (average and range 0.031, 0.001–0.151 mg/kg dw). Interestingly, in the IVD in the presented study the detection of cadmium was confirmed in less than half of the samples, which that should be grounds for further analysis.

**Pb.** A similar situation is observed in lead analysis in which bone concentration is up to 8 times higher – average 1.35 µg/g dw [11]. Cartilage content is smaller – average 0.41 mg/kg and range 0.21–.65 mg/kg [19]. Also, the given range in cartilage analysis – 0.285–1.440 mg/kg dw [16], suggests that lead is the constant component in bone and cartilage, but not in intervertebral disc.

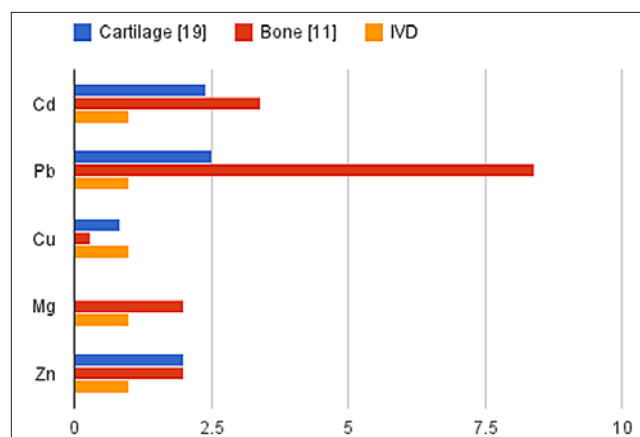
The poorest set of reference data encountered in the literature by the authors was for cobalt, with no possible relationship of its contents to connective tissue. From other studies, it was seen that cobalt was observed in blood serum on a constant basis – range 0.107–4.867 µg/mL [21]. Serum level was also confirmed for cadmium and lead, where in the presented study its presence was confirmed only in a part of the specimens (serum levels ranges – 0.019–1.627 µg/mL and 0.171–8.782 µg/mL, respectively).

Nickel and molybdenum was also determined in a part of the samples. The authors have not encountered in the literature reference data for these TE content in bone, nor in other tissues that might have been helpful in the presented analysis.

As mentioned above, in the current study, Cd, Co, Pb, Mo, Ni were determined only in some samples, whereas studies of bone and cartilage suggest its permanent presence.

**Discrepancy between content of disc trace element compared to bone and cartilage.** This study shows that there is a significant difference in the concentration of elements in bone, cartilage and intervertebral disc (Fig. 1). Interestingly, smaller differences are observed in the essential non-toxic





**Figure 1.** Relative contents of the selected TE in bone and cartilage in reference to IVD (value=1). Values extracted from publication [19] for cartilage and [11] bone

elements Mg and Zn, which have twofold higher contents in cartilage and bone. Cu is the only element with higher concentration to be found in IVD than in bone or cartilage. The differences may be related with the metabolism rate in different areas. Although bone may not be compared with IVD, if the cell concentration and cell/ECM ratio of the IVD are taken into account, some conclusions may be drawn [22]. If it is assumed that TE concentration is mainly related to metabolism and nutrient distribution within the tissues, this suggests that the IVD metabolism rate is  $\frac{1}{2}$  –  $\frac{1}{4}$  slower compared to cartilage. Although the cartilage and bone trace elements are within comparable range, there is no ground to compare their metabolism. Otherwise, comparing the metabolic-related TE concentration in cartilage and bone, it is a far from simple conclusion that the metabolic rate is reflected by the TE contents.

In the presented study, a higher disproportion was observed in the elements related to environmental pollution – Pb and Cd. The much higher concentration in bone and cartilage were probably related mainly to the selective endplate permeability of the IVD [23], especially in that in a part of the samples it was below the level of detection.

The above observation does not match that with the platinum concentration in a cancer patient, which showed a similar or higher concentration in IVD compared to bone tissue [7]. As an explanation, the differences between the analyzed samples have to be considered: exposure characteristics as well as primary disease that may influence the tissue parameters. On the other hand, this study confirms that metal concentration is also higher than in internal organs [8]. Further study of the trace elements may shed new light on the endplate role in IVD degeneration.

**Correlation analysis.** Although the examined group was small, a statistically significant correlation was observed among the elements and age, which has not been observed in previous studies. The positive correlation between Al and age is important information, presented for the first time in this study. For the exogenic source of aluminium there must be considered: oral antacid administration and antiperspirant skin application. Although cooking utensils can affect any cooked foods, aluminum cookware is considered safe, and only uncoated ware may cause leaching of particles into food. Presence of the element in every sample and its correlation with age requires further study.

The strong correlation between Zn and Mg confirms their major metabolic role in the IVD tissue. This correlation was also observed in bone tissue in a population in Cracow, Poland (0.68), by Loska et al. [11]. However, the possibility to link together the metabolic background with TE contents, especially in degenerative process, needs further research.

No other correlation between Mg/Pb, Mg/Cd or Zn/Pb observed in the presented study was observed in bone analysis. The remaining pairs of elements that showed a correlation in this study have not yet been evaluated.

## CONCLUSIONS

To the best of the knowledge of the authors, this study is first to profile the selected trace element sets in intervertebral disc in patients with degenerative changes. The analysis of the presented trace elements in intervertebral disc provides a preliminary picture about its chemical environment and possible dependencies. It may be a new direction for a better understanding of the biological and biochemical changes that take place in the disc. The study confirms that there are significant differences between the trace element concentration in intervertebral disc, compared to the other tissue. It also shows that the presence of aluminum in the disc and its concentration is correlated with age, and the mutual correlation potentially supports the metabolic background of degenerative disc disease. It is also shown that there are pollution-related elements that may be detected in the IVD, although it is the best separated area with no vascularity. This may suggest modification of the end plate permeability to be related with the degeneration process. Whether the degeneration may be linked to pollution related elements, or that those elements may be the markers of dysfunction that may induce the degenerative process needs further study. The data sets presented in the study may be utilized as the reference values in future research.

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